LANTZ

A Study of the Effects of Alcohols upon the Cells of Vicia Faba

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A STUDY OF THE EFFECTS OF ALCOHOLS UPON THE CELLS OF VICIA FABA

BY

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THESIS

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

CYRUS WILLIAM LANTZ

ENTITLED A STUDY OF THE EFFECTS OF ALCOHOLS UPON THE CELLS OF

VICIA FABA

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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Final Examination

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I INTRODUCTION

The effects of various chemical agents upon the plant cell have been studied by a number of investigators. It has been found, as expected, that different chemical agents affect the organs of a cell differently. A clearer understanding of the normal functions of an organ is often made possible through a study of an organ in disease. The action of chloroform, ether, chloral hydrate, phenol, potassium nitrate, copper sulphate, and other reagents have been studied upon various plant cells, but practically no work has been done upon the action of the alcohols.

Czapek (1) in his book entitled "Chemical Phenomena in Life" in a chapter on "The Protoplasmatic Membrane" gives a brief discussion on the toxicity of the alcohols. According to Richardson's law, the higher members of the alcohol series are more toxic than the lower. In other words the toxicity of the alcohols in a series increases with the carbon atoms in the straight chain. Overton's work (2) showed that the toxicity in the alcohol series increased from one member to the next in the ratio of 1 to 3. According to Traube, this is exactly the ratio that exists between the surface tensions of the alcohols.

Bokorny (3) studied the effects of the different alcohols upon infusoria and algae. He found the toxicity increases as one goes up the series, but apparently not in a 1 to 3 ratio. Tsukamoto (4) in his studies upon the effect of the alcohols upon protoplasm obtained similar results. The chief aim of the earlier investigators was to find the fatal dose of the alcohols, and thus to get an idea of their

relative toxicity. Little or no effort has been made to find the effect of the different alcohols upon cell activities, and thus to establish a definite relationship between the chemical structure of the alcohols and the effect upon protoplasm. In the present study, I have attempted to determine whether or not such a relationship exists.

In the time at my disposal it was found possible only partially to fulfill this aim. I have made a number of observations upon the physiological effects of the following alcohols,--methyl, ethyl, propyl, and isobutyl. So far as possible the concentration of these alcohols at which death occurs has been determined. Most of the work, however, has been done on the effects of ethyl and propyl alcohols on the cell. The reason for selecting these is that they were the first to give me definite cytological results, and lack of time did not allow a comparative study of the whole series.

My studies were limited to a consideration of,- general appearance of the root, structure and appearance of the cytoplasm, vacuolization, structure and appearance of the nucleus, the nucleolus, chromatin, chromidia, and mitosis. Only very general observations upon the rate of growth have been made. Much could be done in the correlation of the structure and the action of the cells as influenced by the alcohols.

II MATERIALS AND METHODS

The primary roots of young Vicia faba seedlings were used in this investigation. The seeds were germinated in moist sphagnum which had been sterilized by boiling in order to reduce the growth of fungi. When the roots were from 2 to 4 centimeters in length, they were taken from the sphagnum and placed in tap water for 24 hours to

accustom them to a liquid medium.

The solutions were made from distilled water and chemically pure absolute alcohols which were secured from Dr. Theodor Schuchardt, Görlitz. The alcohols used were,- methyl, CH_3OH; ethyl, CH_3CH_2OH; propyl, $CH_3CH_2CH_2OH$; and isobutyl, $CH_3CH_2CHOH.CH_3$. The solutions were kept in glass jars, and no solution was used a second time.

The method of treatment was as follows: Vicia roots which had been growing in tap water for 24 hours were carefully selected. Some care was taken in order to use only roots which seemed normal in their appearance and growth, and of a uniform length. The roots were then immersed in the various solutions with which they were to be treated. The cotyledons were supported above the solutions by wire netting. At definite intervals roots were removed and fixed immediately; others removed at the same time were returned to tap water for recovery. These latter were fixed at intervals usually of 2 hours and extending over a period of 24 hours. This afforded an opportunity to observe the process of recovery or necrobiosis in any series. Checks were made with each series by fixing normal roots which had been growing in tap water. Some roots were allowed to grow on past the 24 hours in order to see if they continued to live. The temperature of all the solutions and recovery water was kept as nearly constant as possible without using any special device. The temperatures are recorded in the data.

The roots were usually treated at about 11:00 A.M. each day in order to take into account the periodicity of mitosis as determined for Vicia.

Two fixing agents were used:

(1) Picric Acid solution.

Corrosive sublimate - - 5 gr.

Glacial acetic acid - - 5 cc.

Picric acid, a saturated

solution in 50% alcohol - 100 cc.

(2) Chromo-acetic solution

Chromic acid - - - - 6 gm.

Acetic acid - - - - 4 gm.

Water - - - - - - 1000 cc.

The roots were kept in the fixing fluid for 24 hours, washed and dehydrated in the usual way, cleared in chloroform, and infiltrated and imbedded in 52° paraffin.

The sections were cut 9 microns in thickness and were stained by the Haidenhain iron-alum-haematoxylin method. They were mordanted for 3 hours, stained about 18 hours, differentiated under a microscope, dehydrated, cleared in cedar oil, and mounted in balsam.

III GENERAL RESULTS

The concentration at which the primary root is killed was determined for each alcohol.

1. E 1. Ethyl Alcohol

A ten-minute treatment with 20% ethyl alcohol killed all of the roots.

A 10% solution was then used with the following results:

Time	Temperature	No. of series	Results
10 minutes	20° - 22°	2	All recovered
20 "	" "	3	" "
30 "	" "	3	Nearly all recovered
40 "	" "	2	Most roots killed

Another series in which the roots were more active seemed to be more susceptible to the alcohol. The temperature in this case was a little higher, 22°- 24°. Results of this series are:

20 minute treatment - - - 3 out of 4 recovered

30 " " - - - 3 " " 5 "

40 " " - - - All were killed.

The roots that died usually lost their turgidity after 6 or 8 hours in the recovery water, and death took place at about the same time. A constirction often formed about 4 or 5 millimeters from the tip of the root, and the tip soon died.

In all of these cases the ethyl alcohol retarded the growth of the roots.

2. Propyl Alcohol

All roots were killed with a 10% solution of propyl alcohol when treated for only 10 minutes.

A 5% solution of propyl alcohol gave the following results:

Time	Temperature	No. of series	Results
10 minutes	20°- 22°	2	All recovered
20 "	" "	3	" "
30 "	" "	3	" "
40 "	" "	2	All recovered, but many dead cells in periblem

With a 6% solution a treatment of 30 minutes killed about one-third of the roots.

The propyl alcohol also retarded the growth of the roots. A forty-minute treatment with 5% propyl alcohol seemed to have about the toxic effect of a twenty-minute treatment with 10% ethyl alcohol.

3. Isobutyl Alcohol

A 5% solution after a ten-minute treatment killed all of the

roots. A 3% solution after a ten-minute treatment killed most of the roots. A 1% solution after a forty-minute treatment killed none, and the roots grew about normally. The critical concentration of isobutyl alcohol for a treatment of from 10 to 40 minutes is close to 2%.

4. Methyl Alcohol

A 15% methyl alcohol killed all the roots when they were treated for only 10 minutes. A treatment of 10 minutes with 10% methyl alcohol killed a number of the roots, while a thirty-minute treatment killed all. The results here obtained seem to indicate that the methyl alcohol is slightly more toxic than the ethyl. This is contrary to what one would expect. The work with the methyl alcohol has not been very extensive, and further work should be done.

IV CYTOLOGICAL RESULTS

1. Ethyl Alcohol

Sections of roots which had been treated in ethyl alcohol for 10, 20, 30, and 40 minutes, and the recoveries for these treatments for intervals of ½. 1, 2, 4, 6, 8, 10, 12, 14, and 24 hours were studied. Those treated for 40 minutes died so they were observed only to study the changes leading to death. The twenty-minute treatment gave the most interesting results. The following data is a summary of the cytological effects of the ethyl alcohol.

Cytoplasm: The abundant cytoplasm appears distinctly granular and rather dense immediately after treatment. This appearance and condition is retained during the first 2 hours of recovery. Beginning with a four-hour recovery, the cytoplasm becomes less dense, more vacuolar, and while the granular appearance remains, the tendency is for these granules to collect in bunches or knots. The cyto-

plasm remains less dense for sometime, but unless the treatment has been very severe, it regains its original structure within 24 hours.

The period in the recoveries extending from 4 to 6 hours is a very critical period in the recovery of the cells. Death, if it occurs at all, takes place near the six-hour period. During this period much chromatin in the form of granules is present in the cytoplasm. This is especially true of cells of the inner periblem. Large irregular masses of chromatin are found very abundant in the cytoplasm.(Fig. 4). These are of the same size, shape, and general appearance as the chromatin masses found on the reticulum of the nucleus at this time. The most striking structures noticeable at this time are very abundant, dark-staining, thread-like bodies in the cytoplasm (Figs. 3, 5, 7). They appear as long threads staining dark like chromatin. Some are almost straight, some are wavy, others are coiled like coiled springs. I can find no connection between these and the chromatin found in the nucleus. Other dark staining bodies are found in the cytoplasm. These, judging from their light centers, are hollow or vacuolar (Fig. 6). All stages between the solid bodies and hollow vesicles are found. This suggests a corrosion of the chromatin masses, chromidia, thrown off from the nuclei.

Nuclei, nucleoli and chromidia: The nuclei immediately after treatment generally show but little chromatin on the reticulum, and what is there is usually in the form of fine granules (Fig. 1). The fine chromatin particles give the nuclei a homogeneous appearance. The nuclear membrane is not distinct, and consequently the nuclei are not clearly differentiated from the cytoplasm, especially when the cytoplasm is very dense. The nucleoli are rather large, fairly regular in outline, and show little or no hof.

Beginning with the two-hour recovery changes take place in the

nuclei and nucleoli. The nucleoli become irregular and jagged in shape. The hof entirely disappears. There is an abundance of chromatin in the nuclei, and it usually collects on the reticulum in large masses causing them to lose their homogeneous appearance. The ground substance stains light, and the dark chromatin granules stand out in sharp contrast to it. The nuclear membrane stains very distinctly, probably due to the collecting of chromatin against the membrane.

This chromatin material of the nuclei seems to be thrown off into the cytoplasm in large quantities in the four- and six-hour recoveries. This conclusion is based on these facts: (1). The nuclei become irregular in outline. The chromatin masses collect on the nuclear membrane and apparently push it out so as to form projections or papillae (Fig. 4); (2) chromatin masses of the same size and shape are found just outside of the nuclei in the cytoplasm (Fig. 4); (3) in the eight-hour recovery there is not so much chromatin on the reticulum.

If the root is seriously affected, but yet continues to live, the cells in the recoveries 10, 12, 14 and 24 hours will show small nucleoli and a small amount of cytoplasm (Fig. 2). Chromatin will not be very abundant. The hof usually appears after about 10 hours recovery. Roots not so badly affected recover a little sooner, and after 24 hours they appear about normal.

Mitosis: The nuclei continue to divide about normally to the period of chromatolysis at the four- and six-hour periods of recovery, then if the treatment has been very severe, fewer divisions are seen. The spindles for the most part are normal. An occasional deranged spindle, usually a late anaphase, is found. In such cases the spindle fibers are broken and thrown to one side, and the chromosomes are

scattered. In general, the mitotic figures are little affected.

Disintegration: Death when it occurs usually takes place about 6 hours after treatment. The tip cells and the cells of the periblem are the first to die. The changes leading to death are somewhat as follows: The cytoplasm becomes more vacuolated, the nucleoli usually break up, chromatin collects on the reticulum in large masses and is finally extruded into the cytoplasm. There is a decided chromatolysis. In the periblem, the nuclei are usually pushed out and flattened against the cell walls by the vacuoles. The whole nucleus soon stains jet black.

2. Propyl Alcohol

Cytoplasm: The cytoplasm throughout the treatments has a flaky, flocculent appearance. It does not have the distinctly granular appearance found in the treatments with the ethyl alcohol.

Immediately after the twenty- and thirty-minute treatments and for one hour during recovery, the cytoplasm is dense and abundant (Fig. 11). Beginning with the two-hour recovery and extending over a period of 10 or 12 hours, it is not so dense, vacuoles are abundant, and it collects in knots or masses. After 12 hours the cytoplasm usually again becomes more abundant and more dense.

The cytoplasm in roots treated for 40 minutes has the same general appearance as the cytoplasm of the roots after a two-hour recovery from the twenty- and thirty-minute treatments. It does not resume its normal structure even after 24 hours of recovery. In these late recoveries the cytoplasm is not dense nor abundant, and there are many vacuoles (Fig. 12).

In no case do I find the peculiar thread-like bodies which are so abundant in the roots treated in ethyl alcohol. Chromidia are

found in the periblem, but there is not so much granular chromatin in the cytoplasm. The peculiar vacuolar bodies described in the roots treated in ethyl alcohol are also found here. They appear at the time, or perhaps a little later, that the chromidia become most abundant. At the six-hour recovery period in the roots treated for 30 minutes these bodies are very abundant. In the eight-hour recovery they are not so abundant, and they stain less deeply. They become fainter and fainter, and at the twelve-hour period they have disappeared. The cells of the outer periblem are usually full of them, although they are also found in the cells of the inner periblem (Figs. 8, 9, 10).

Nuclei, nucleoli, and chromidia: The nuclei in the twenty- and thrity-minute treatments are usually poor in chromatin (Fig. 11). Beginning with the one-hour recovery the chromatin becomes more abundant and collects on the reticulum in rather large masses. In the forty-minute treatment the nuclei show this immediately after treatment. The chromidia appear a little sooner in the roots treated with the propyl than with the ethyl alcohol. The general appearance of the nuclei at the corresponding stages is about the same with the two alcohols. The nuclear membrane has the same irregular appearance, and the chromatin masses--chromidia--are being extruded from the nuclei into the cytoplasm (Fig. 8). The time of their appearance varied more with the propyl than with the ethyl alcohol. They are most abundant in roots treated for 20 and 30 minutes after a recovery of from 2 to 6 hours. The roots with the forty-minute treatment show them most abundantly immediately after treatment. The chromidia gradually become smaller, often vacuolar, and soon disappear. They seem to be used up in the cellular activities.

The nucleoli immediately after treatment are usually large and

irregular (Fig. 11). The hof at this time has disappeared. This condition prevails up through the period of chromatolysis. Immediately after this period many of the nucleoli are small. Nucleoli of roots treated for 20 or 30 minutes soon become normal. The hof appears after about 8 hours of recovery. In the forty-minute treatment the nucleoli are still small after 24 hours (Fig. 12). They are they regular in outline and are surrounded by a large hof.

Mitosis: The mitotic figures in the roots with all the treatments seem to be normal. During the period of chromatolysis, the mitotic process is checked. At the eight-hour recovery period there are very few divisions. Cells in roots treated for 20 minutes commence dividing again at the ten-hour period and continue to do so normally. Cell division in roots treated for 30 and 40 minutes is rare even after 24 hours of recovery.

3. Isobutyl Alcohol

No conclusive results were obtained from the roots treated in isobutyl alcohol because only a few observations were made. Most of the roots treated with a 3% solution for 10 minutes died. Death occurred after 4 or 6 hours. These roots show the cytoplasm collected in knots as described before. There is also a marked chromatolysis. The cytoplasm in the cells of the inner periblem show the peculiar thread-like bodies and the hollow vesicles which have been described. Many more series would have to be studied before definite conclusions could be made. These observations, however, seem to indicate a few points of similarity between the effects of isobutyl alcohol and ethyl and propyl alcohol.

4. Methyl Alcohol

No conclusive cytological results were obtained with the roots treated in methyl alcohol. As stated before, most of the roots

treated for the periods indicated died. My observations suggest the following:

The roots treated with 10% methyl alcohol for 10 minutes show the cytoplasm to be similar to that described in the roots treated in ethyl alcohol. In the six-hour recovery, the hollow vesicles so abundant in the roots treated in ethyl and propyl are evident. There is a slight chromatolysis, but not so much as after the treatment with ethyl alcohol. The mitotic processes seem to be checked.

In the more severe treatments leading to death all the cellular processes are apparently stopped at once. Very little chromatin is evident on the reticulum, and the cells die without any chromatolysis.

V DISCUSSION AND SUMMARY

The alcohols all have a marked toxic effect. The concentrations used were close to the critical concentrations. Davenport (5) classes the alcohols among the catalytic poisons, which according to Nageli are poisonous "by virtue of an inherent molecular movement which disturbs the normal condition of movement in the living plasma, and on that account produces death". Loew (6) believes, more precisely, "that the transmitted condition of violent movement leads to chemical transformations in the unstable albumen of the protoplasm".

My results show a relation between the molecular structure of the alcohols and their toxicity. The toxicity of the ethyl, propyl, and isobutyl alcohols increases up the series with the molecular weight. Using a twenty-minute treatment, the following concentrations of alcohols have about the same degree of toxicity,- 10% ethyl, 6% propyl, and 2% isobutyl. There seems to be a fairly constant difference of 4% between these concentrations. My results do not show the ratio of toxicity from one member to the next to be 1 to 3.

My observations upon the toxicity of the methyl alcohol would indicate that it does not fit well into this series. The critical concentration of the methyl alcohol seems to be about the same as that of the ethyl alcohol. It even is slightly more toxic, though its effects have not been fully investigated. It was the last one taken up, and lack of time has prevented a complete investigation. It is possible that the methyl alcohol contained some impurity, such as fusel oil, which would account for its unusual toxicity. Only one stock solution was used.

The alcohols at the concentrations used in all cases retarded the growth of the roots.

The critical period in the recovery of the roots from the treatments as already discussed is a period of from 4 to 6 hours following treatment. This is the period when the cells are making their most active efforts to recover. If death occurs, it usually takes place at this time.

The cytoplasm is similarly affected in the different alcohols. It is very dense immediately after treatment, but during the critical period of reconstruction, it becomes vacuolar, and collects in knots or bunches. It is less dense at this time, and if the treatment has been very severe, it will continue to be thus for 24 hours. Part of the trophoplasm is used up and a clear, glassy structure is left. This seems to indicate that the cytoplasm is used up in the metabolic processes incident to recovery. The cytoplasm of the roots treated in the methyl and the ethyl alcohols seems to be more granular than that of the roots treated in the other alcohols.

There are three kinds of bodies found in the cytoplasm,- thread-like bodies, solid dark-staining bodies, and hollow vesicles.

The thread-like structures are found only in the roots treated in ethyl alcohol and in those treated in 3% isobutyl alcohol. Those treated in the isobutyl alcohol died, so it was impossible to determine the fate of the threads. The threads in the roots treated in the ethyl alcohol soon disappear. No connection between these threads and the nuclei is found, and it seems that they are of cytoplasmic origin. Similar bodies--mitosomes--have been described by Matthews (7) in the pancreas cells of Necturus. He thinks that they are of cytoplasmic origin, and that they are directly connected with the peripherally placed chromatin of the nuclei. According to Matthews, the chromatin acts as a catalytic agent in the metabolism of the cell, and the mitosomes disintegrate to form zymogen granules. I could not find the nuclear connection that he describes. Others have called similar bodies mitochondria.

All of the alcohols caused the appearance in the cytoplasm of solid bodies and hollow vesicle-like bodies. These solid bodies have been called chromidia, chondriosomes, and various other names. They appear in nearly every case about 4 or 6 hours after treatment. They are found a little sooner in the roots treated in propyl alcohol than in those treated in ethyl alcohol. They seem to be chromatin masses that are extruded from the nuclei. Immediately after treatment the nuclei usually show but little chromatin. Soon, however, the chromatin commences to collect in rather large masses on the reticulum and against the nuclear membrane. The nuclei become irregular owing to the formation of the papillae by the pressure of the chromatin. At this period and immediately following it, chromatin masses are found abundantly in the cytoplasm. They gradually disappear and are not found in the later recoveries. The process causing their disap-

is a sort of a corrosion, supposedly by an enzyme. The central part is usually the first to disappear. This gives to them a vacuolar appearance, and seems to account for the hollow vesicles above described. Some chromidia do not become vacuolar and seem to be attacked from the outside. This is especially true of the chromidia in the outer periblem of the roots treated in propyl alcohol. Similar bodies in the cytoplasm are described by Lewitsky (8) in Albugo, by Wager (9) in Polyphagus Euglenae, and by Moroff (10) in the eggs of Copepoda. Moroff thinks that they are used to form yolk. Wager says chromidia are extruded for metabolism, and that the vacuolar appearance of some of them is due to the changing of the chromatin into fat globules.

My observations upon the cells of Vicia faba indicate that the chromidia are extruded from the nuclei and are used directly in metabolism. I can see no evidence for believing that the chromatin is catalytic in function. The period when the chromidia are extruded is the most critical period in the recovery of the cells. It is a period when the metabolic processes are very active in the attempt to overcome the poison. If the roots have not been too severely treated, they soon become normal after the disappearance of the chromidia. During this critical period there is a decrease in the amount of cytoplasm undoubtedly due to the great activity of metabolism. My observations would lead me to agree with Wager's interpretation of the function of chromidia.

There seems to be some relationship between the extrusion of chromidia and the nucleoli. Just before the extrusion of the chromidia, the hof disappears and the nucleoli become irregular and jagged. If the treatment has been very severe, the nucleoli are smaller after the throwing out of the chromatin. In the cases where the

treatment is not so severe, the nucleoli only slightly decrease in size, and chromatin continues to be formed on the reticulum. This suggests that the nucleoli have something to do with the formation of chromatin which is extruded as chromidia.

The mitotic process is checked by the various alcohols. Practically all division is stopped during the critical period of the recovery. If the treatment is not very severe, the recovery is rapid, but if severe,there are few mitotic figures even after 24 hours. A few deranged spindles in the late anaphase stage are found in the roots treated in ethyl alcohol. In general, I should say that there is very little effect on the spindles.

In case of the death of the root, there is usually a marked chromatolysis. The one exception to this is in the roots treated in 15% methyl alcohol. Here the cells died without much chromatin being formed and without chromatolysis.

I may say in summing up that the ethyl and propyl alcohols have similar effects upon the cellular activities of Vicia faba. The difference between their effects is one of degree rather than of kind, the propyl alcohol being the more toxic. My observations upon the effects of methyl and propyl alcohols are not complete enough for me to draw definite conclusions, but they do not seem to show a different kind of effect. The only other differences between the effects of the ethyl and the propyl alcohols arethat the ethyl gives the cytoplasm a granular appearance, and produces thread-like structures in the cytoplasm. Neither of these conditions is found in the roots treated in the propyl alcohol. The alcohols seem particularly to affect the metabolic processes of the cells.

Much work remains to be done on this problem. The effects of methyl and isobutyl alcohols should be carefully studied. Weaker

solutions of all the alcohols should be used for varying periods. The rate of growth and the cellular activities should be carefully correlated. Lastly, the toxicity of the alcohols in relation to their surface tensions should be investigated.

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EXPLANATION OF PLATES

Plate I. Treatment with 10% ethyl alcohol. (x1200)

Fig. 1. Treated for 20 minutes; no recovery. Shows dense cytoplasm, small amount of chromatin on reticulum, large nucleoli.

Fig. 2. Treated for 30 minutes; 24 hours recovery. Shows less cytoplasm, smaller nucleoli.

Figs. 3, 5, 7. Treated for 20 minutes; 6 hours recovery. Shows irregular nuclei, thread-like structures in the cytoplasm.

Fig. 4. Treated for 20 minutes; 4 hours recovery. Shows irregular nuclei, chromatin on reticulum, extrusion of chromatin, and appearance of hollow vesicles.

Plate II. Treatment with 5% propyl alcohol. (x1200)

Fig. 8. Treated for 20 minutes; 4 hours recovery. Shows irregular nuclei, extrusion of chromatin, chromidia.

Fig. 9. Treated for 30 minutes; 6 hours recovery. Shows chromidia and hollow vesicles.

Fig. 10. Treated for 30 minutes; 8 hours recovery. Hollow vesicles becoming fainter.

Fig. 11. Treated for 20 minutes; no recovery. Shows dense cytoplasm, small amount of chromatin on reticulum, and large irregular nucleoli.

Fig. 12. Treated for 40 minutes; 24 hours recovery. Shows small amount of cytoplasm and chromatin, very small nucleoli, and large hof.

Fig 1.

Fig. 2

Fig. 3

Fig 4

Fig. 5

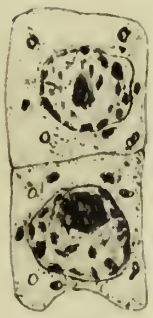

Fig 6.

Fig. 7

Plate I

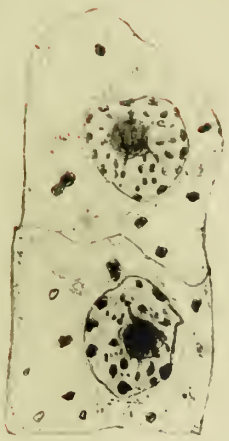

Fig. 8

Fig. 9

Fig. 10

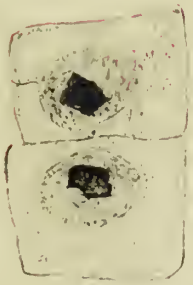

Fig. 11

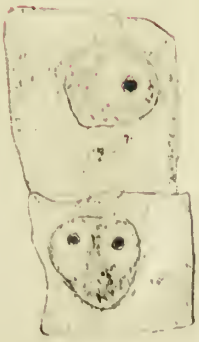

Fig 12

Plate II